Journal of Surgical Oncology 1998;68:25–29

Different Distributions of Immunoreactive S100-α and S100-β Protein Expression in Human Breast Cancer

HIROOMI FUNAHASHI, MD,[1]* TAKASHI KOSHIKAWA, MD,[2] SHU ICHIHARA, MD,[3] EKOH OHIKE, MD,[1] AND KANEHUSA KATOH, MD[4]

[1]Department of Surgery II, Nagoya University School of Medicine, Nagoya, Japan
[2]Department of Pathology and Clinical Laboratories, Aichi Cancer Center Hospital, Nagoya, Japan
[3]Department of Pathology, Nagoya National Hospital, Nagoya, Japan
[4]Department of Biochemistry, Institute for Developmental Research, Aichi Prefectural Colony, Nagoya, Japan

Background and Objectives: Although the localization of S100 protein in breast carcinoma has previously been studied, the immunohistochemical expression of the S100-α and -β subunits has not been examined.
Methods: Immunohistochemical staining for S100-α and S100-β proteins was performed on 72 benign breast lesions and 72 infiltrating ductal carcinoma of the breast. Noncross-reactive anti-S100-α and anti-S100-β antibodies purified by affinity chromatography were used in the studies.
Results: More than 30% of the epithelial cells comprising all the benign lesions were either S100-α or S100-β positive. In breast carcinoma cases, however, >30% of malignant cells were S100-α positive in 70/72 cases (97.2%), whereas the number of S100-β positive cells exceeded 30% in only 3/72 cases (4.0%).
Conclusions: Immunohistochemical staining for S100-α and S100-β proteins may help to differentiate benign proliferative breast lesions from breast cancers in difficult cases.
J. Surg. Oncol. 1998;68:25–29.

KEY WORDS: **breast cancer; S100-α and S100-β protein; immunohistochemistry; benign breast disease**

INTRODUCTION

The S100 protein, molecular weight 21000, discovered by Moore et al. [1,2] is an acidic calcium-binding protein. S100 protein is mainly found in Schwann cells, melanocytes, adipocytes, and Langerhans cells [3,4]. However, these proteins also have been found in both glandular epithelial cells and myoepithelial cells of the mammary glands. S100 is of extremely stable antigenicity and therefore, can be readily detected using ordinary paraffin sections. Furthermore, S100 protein expression is not affected by changes in the cell cycle. These characteristics make this protein an effective marker in pathologic diagnosis. S100 protein is a dimer composed of α chain and β chain subunits, which, depending on the particular subunit combination, exists in three forms: S100ao (α α), S100a (α β), and S100b (β β) [5]. Haimoto et al. [6,7] have used noncross-reactive anti-S100-α and anti-S100-β antibodies purified by affinity chromatography to study the immunohistochemical localization of S100-α and S100-β in various tissues [6,7].

S100 protein expression also has been identified in breast carcinoma because of the inherent stability of the S100 protein [8–11]. These studies did not specifically examine expression of the α and β subunits. In the present study, we used antibodies previously employed by Haimoto et al. [6] to compare the immunohistochemical expression of S100 subunits in benign and malignant breast tissues.

*Correspondence to: Dr. Hiroomi Funahashi, Department of Surgery II, Nagoya University School of Medicine, 65, Tsurumai-cho, Showa-ku, Nagoya 466, Japan. Telephone No.: (81) 52-744-2245; Fax No.: (81) 52-744-2255. E-mail: hfunahas@tsuru.med.nagoya-u.ac.jp
Accepted 17 February 1998

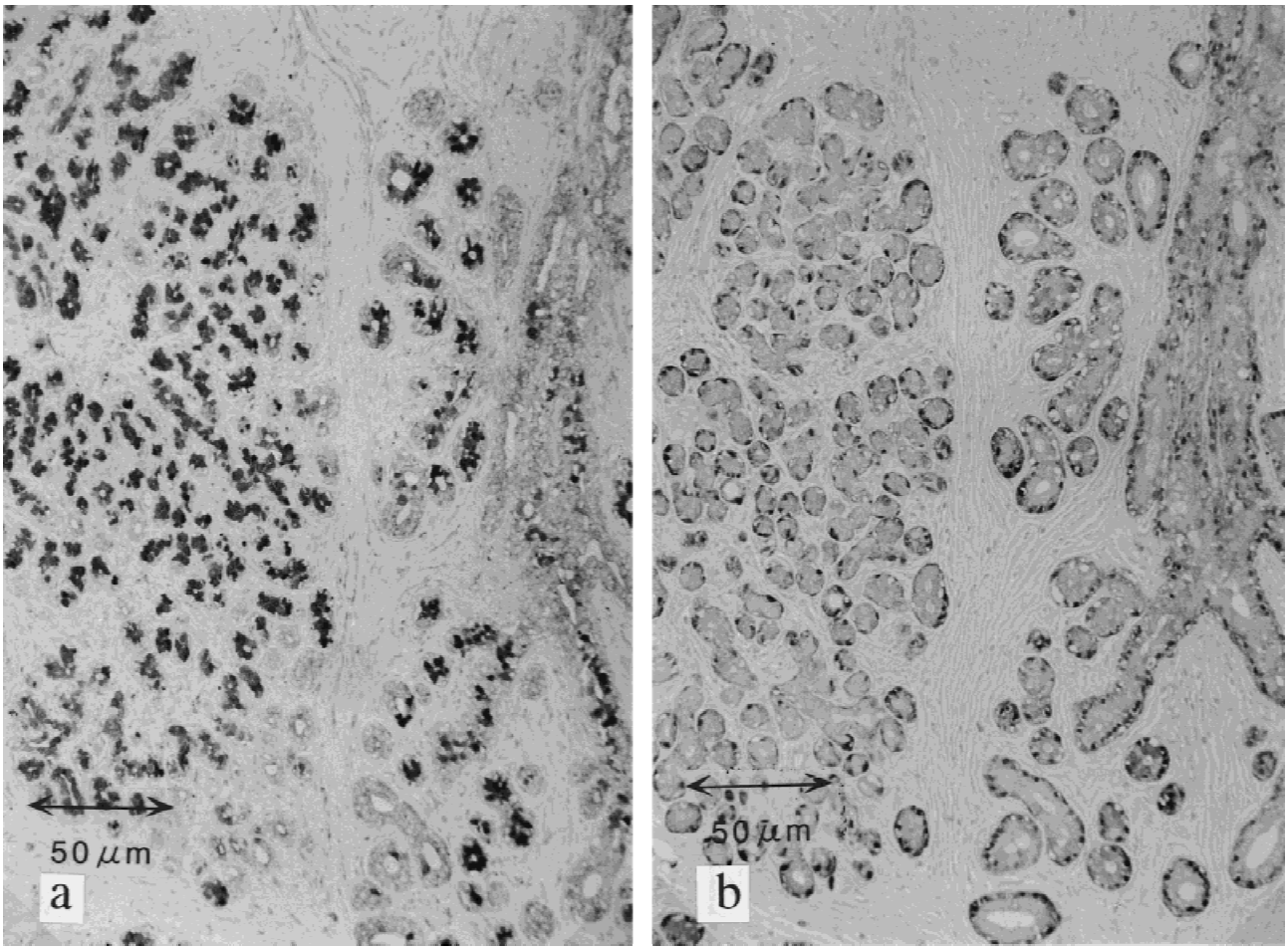

Fig. 1. Immunohistochemical distribution of S100-α and S100-β positive cells in normal breast tissue. **(a)** S100-α, **(b)** S100-β. S100-α is localized mainly in terminal duct epithelial cells and lobular cells, and also found in myoepithelial cells. S100-β is localized mainly in myoepithelial cells and only rarely seen in duct epithelial cells (×100).

MATERIALS AND METHODS

Seventy-two invasive breast ductal carcinomas and 72 benign breast lesions (fibrocystic disease, 34 cases; fibroadenoma, 38 cases) removed between 1989 to 1993 were studied. No unusual types of breast carcinoma were included. Five-micron sections were cut from paraffin blocks, and immunohistochemical staining was performed using streptavidin-biotin kit (Vector, Burlingame,CA). Primary rabbit antihuman antibodies (anti-S100-α and anti-S100-β) were raised in our laboratory. Secondary antirabbit antibodies (MBL Co.,Tokyo, Japan) were labeled by peroxidase [12]. Adipocytes provided a built-in control for both subunits of S100 protein, whereas peripheral nerve fibers provided a positive control for S100-b subunit. Rat immunoglobulin was substituted for the primary antibody as a negative control. No reaction was observed in the negative controls. Staining was evaluated by only one pathologist (T.K., co-author), in a semiquantitative fashion: cases in which >30% of the epithelial cells forming the lesion were reactive were rated +, 10–30% reactive were ±, and cases with 10% reactive were −.

RESULTS

Localization of S100-α and S100-β Proteins in Normal Mammary Tissue

S100-α and S100-β localization in the normal epithelium was studied in adjunctive normal mammary tissue surrounding benign and malignant samples removed surgically. The majority of ductal epithelial cells examined were S100-α– and S100-β–negative or only weakly positive. The intra- and extra-lobular terminal duct epithelial cells and lobular cells were strongly S100-α–positive and S100-β–negative or weakly positive. The myoepithelial cells were S100-α–positive to weakly positive and were strongly S100-β–positive (Fig. 1). Thus the S100 protein subunits found in the terminal duct epithelial cells were largely of the S100ao (α α) type, whereas those seen in

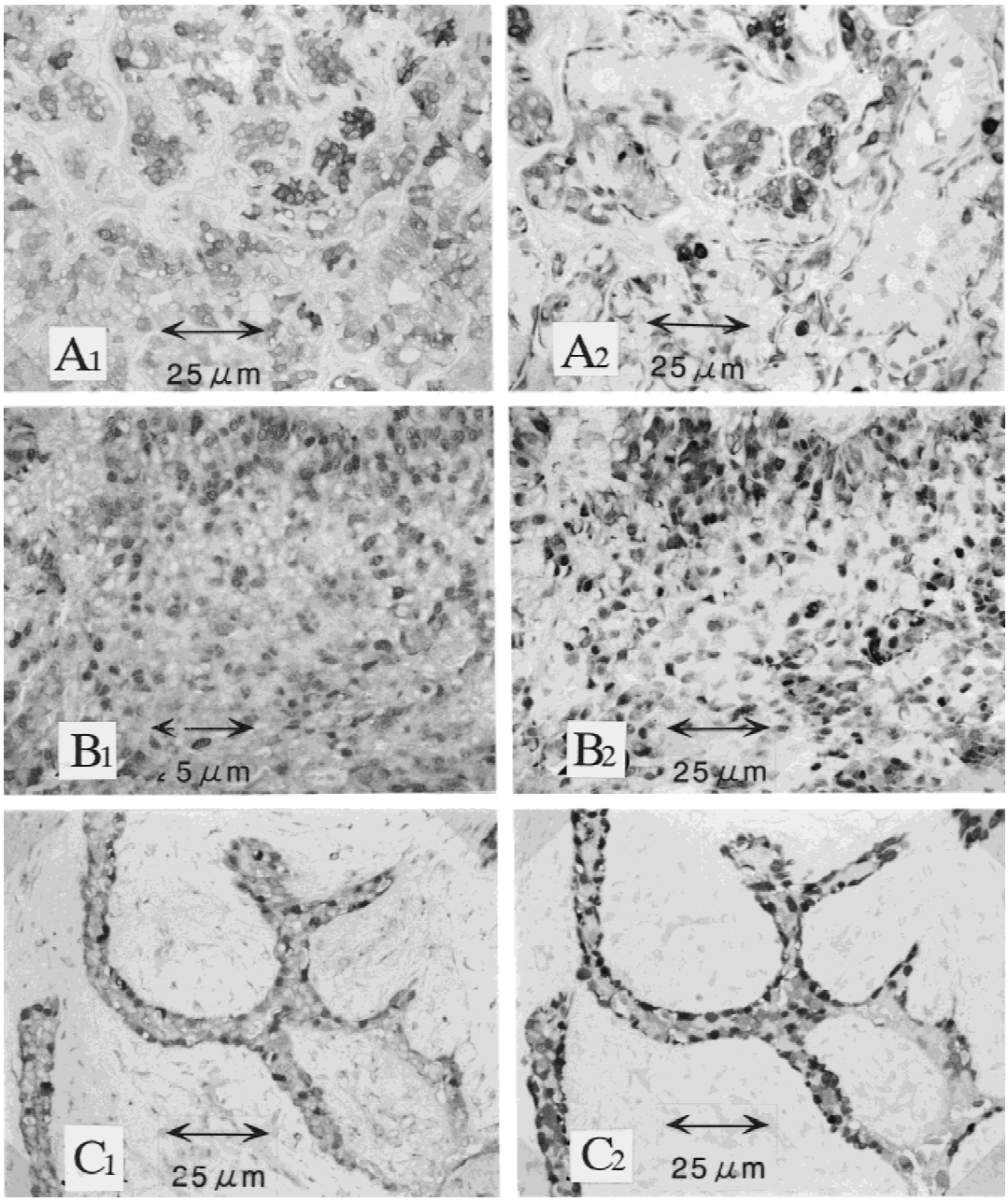

Fig. 2. Immunohistochemical distribution of S100-α and S100-β positive cells in benign breast lesions. (A1,B1,C1) S100-α, (A2,B2,C2) S100-β, (A1,A2) sclerosing adenosis, (B1,B2) intraductal hyperplasia (epitheliosis), (C1,C2) fibroadenoma. Both S100-α and S100-β positive cells are present in >30% of the epithelial cells in all benign breast lesions (×160).

TABLE I. Immunohistochemical Staining of S100-α and S100-β Protein in Benign and Malignant Breast Lesions

	S100-α			S100-β		
	−	±	+	−	±	+
Benign lesion	0	0	72	0	0	72
Invasive ductal carcinoma	2	0	70	54	15	3

the myoepithelial cells they were mainly of the S100b (β β) variety.

Localization of S100-α and S100-β Proteins in Benign Breast Disease

In the proliferative portions of fibrocystic disease and fibroadenoma samples, there was a mixture of S100-α– and S100-β–positive cells. Various types of staining were observed in sclerosing adenosis and intraductal hyperplasia (epitheliosis) in fibrocystic disease and in a

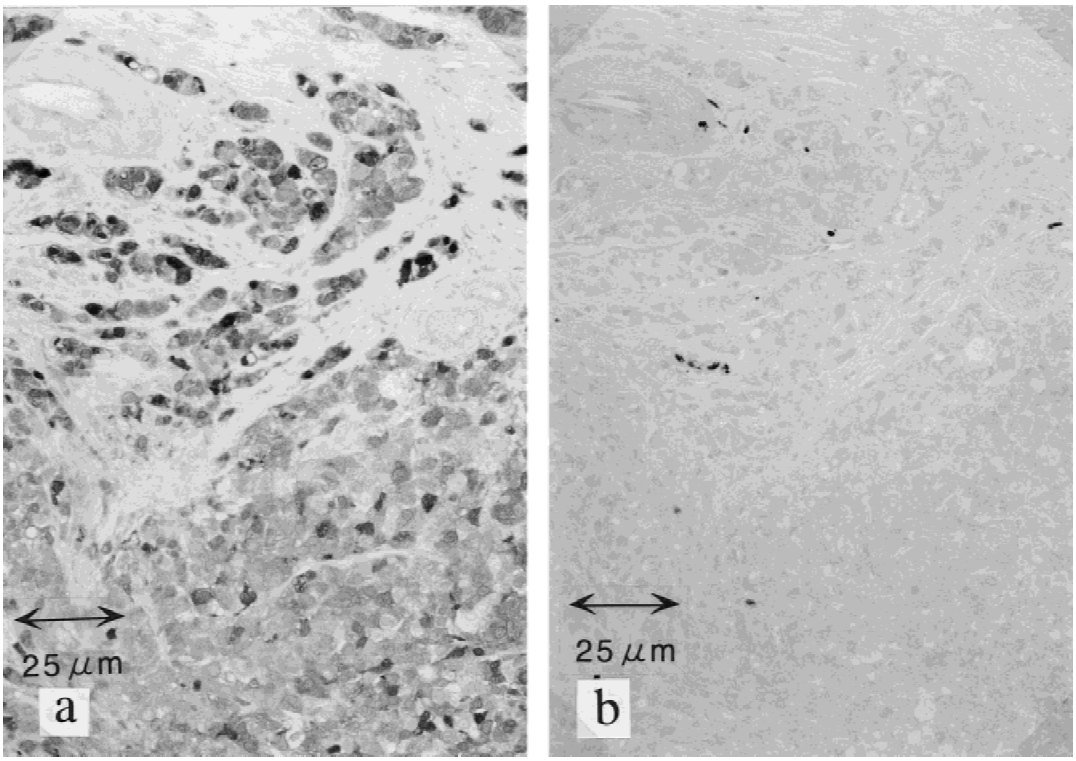

Fig. 3. Immunohistochemical distribution of S100-α and S100-β positive cells in invasive breast carcinoma . **(a)** S100-α, **(b)** S100-β. Most cancer cells are strongly S100-α positive, but almost no malignant cells are S100-β positive. Only a few S100-β positive cells are observed in peripheral nerves (×160).

fibroadenoma case (Fig. 2). In all of these benign lesions, both S100-α– and S100-β–positive cells comprised >30% of the epithelial cells examined. The positive and negative cells formed a mosaic pattern. This feature was especially prominent in areas of intraductal hyperplasia with marked cell proliferation. These findings suggest that the numbers of both S100ao (α α) and S100b (β β) type cells, or of S100a (α β) type cells, are increased in benign breast disease with epithelial cell proliferation.

Overall, both S100-α and S100-β expression was found in all 72 benign breast disease cases (Table I).

Localization of S100-α and S100-β Proteins in Invasive Ductal Carcinoma

Almost all tumor cells comprising the ductal carcinomas were S100-α–positive and S100-β–negative (Fig. 3). Overall, S100-α was expressed in 70 of 72 invasive carcinomas (97.2%), whereas S100-β was rated + in only 3 of 72 tumors (4.2%) (Table I).

DISCUSSION

S100 protein expression has been previously studied in breast carcinoma using antibodies raised against an S100 protein mixture isolated from bovine brain composed mostly of S100a and S100b types. S100ao accounts for only a small percentage of the total S100 protein found in brain. Therefore, S100 protein-positive rates in breast carcinomas have ranged from 10–45% [8–11]. However, anti-S100-α and anti-S100-β antibodies eluted by affinity chromatography are highly pure and not cross-reactive. In this report, we studied the localization of S100-α and S100-β subunits in breast tissue using these highly specific antibodies.

In the normal mammary gland, mammary duct epithelial cells and myoepithelial cells generally form a two-layer arrangement. This feature is maintained in benign proliferative disease and loss of the double cell layer helps distinguish benign from malignant disease. Nakamura et al. [13] used actin as a marker for myoepithelial cells and found that normal breast tissue or benign disease contained a layer of myoepithelial cells. Although such a layer can be found in the intraductal proliferating region of malignant diseases, the myoepithelial cell is lost in invasive extraductal carcinomas [13]. Although not as specific as actin, S100-β was particularly useful as a myoepithelial cell marker. Therefore, the finding that S100-β was positive in benign disease and negative in breast carcinoma was not unexpected. Nevertheless, S100-β is also expressed in glandular epithelial cells other than myoepithelial cells so that the S100-β negativity in breast carcinoma cannot be entirely explained based on the absence of a myoepithelial cell layer. Be-

nign lesions are heterogeneous in that proliferating cells can be either S100-α– or S100-β–positive. Following cancerization, only S100-α–positive cells may remain. From detailed histologic examination, Ohuchi et al. [14,15] concluded that extralobular mammary and intralobular mammary ducts, the terminal duct lobular unit (TDLU), is an important point of origin for breast carcinomas. In the present study, S100-α was mainly localized in the mammary duct epithelial cells that formed the TDLU of normal mammary glands. In breast carcinoma samples, almost all tissue was S100-α–positive. This result implies that the immunohistochemical characteristics of breast carcinomas are similar to those of terminal duct cells.

Based on our findings, S100-α and S100-β expression was very common in intraductal papillomatosis and other benign proliferative disease. These trends may help to distinguish benign disease from carcinoma. Ichihara et al. [16] performed S100-α, S100-β, and actin immunohistochemical staining in 14 cases of epitheliosis and 32 ductal carcinomas in situ (DCIS). Neither epitheliosis nor DCIS expressed actin in the proliferating epithelial cells. In epitheliosis, however, S100-α and S100-β staining formed a mosaic pattern, whereas DCIS only expressed S100-α [16]. Portions of the proliferating areas of benign mammary duct epithelial cells may sometimes lack myoepithelial cell proliferation. In such cases, actin immunostaining alone cannot definitely distinguish benign and malignant epithelium. However, our findings show that a mosaic pattern of S100-α and S100-β immunostaining is characteristic of benign disease. Malignant cases were positive only for S100-α. Thus, S100-α and S100-β immunostaining can help to distinguish benign from malignant breast epithelium.

CONCLUSIONS

Immunohistochemical staining using anti-S100-α and anti-S100-β antibodies may be helpful in the histopathologic diagnosis of breast epithelial lesions in cases where hematoxylin and eosin staining alone cannot distinguish benign from malignant disease.

REFERENCES

1. Moore BW: A soluble protein characteristic of the nervous system. Biochem Biophys Res Commun 1965;19:739–744.
2. Moore BW, McGregor D: Chromatographic and electrophoretic fractionation of soluble proteins of brain and liver. J Biol Chem 1965;240:1647–1653.
3. Nakajima T, Kameya T, Watanabe S, et al.: An immunoperoxidase study of S100 protein distribution in normal and neoplastic tissues. Am J Surg Pathol 1982;6:715–727.
4. Takahashi K, Isobe T, Ohtsuki Y, et al.: Immunohistochemical study of the distribution of α and β subunits of S100 protein in human neoplasm and normal tissues. Virchows Arch (Cell Pathol) 1984;45:385–396.
5. Isobe T, Ishioka N, Okuyama T: Structural relation of two S-100 proteins in bovine brain: Subunit composition of S100a protein. Eur J Biochem 1981;115:469–474.
6. Haimoto H, Hosoda S, Kato K: Differential distribution of immunoreactive S100-α and S100-β proteins in normal nervous human tissues. Lab Invest 1987;57:489–498.
7. Kato K, Kimura S, Haimoto H, Suzuki F: S100ao (α α) protein: Distribution in muscle tissues of various animals and purification from human pectoral muscle. J Neurochem 1986;46:1555–1560.
8. Gillet CE, Bobrow LG, Millis RR: S100 protein in human mammary tissue: immunoreactivity in breast carcinoma, including Paget's disease of the nipple, and value as a marker of myoepithelial cells. J Pathol 1990;160:19–24.
9. Matsushima S, Mori M, Adachi Y, et al.: S100 protein positive human breast carcinomas: An immunohistochemical study. J Surg Oncol 1994;55:108–113.
10. Dwarakanath S, Lee AKC, Delellis RA, et al.: S-100 protein positivity in breast carcinomas: A potential pitfall in diagnostic immunohistochemistry. Hum Pathol 1987;18:1144–1148.
11. Stroup RM, Pinkus GS: S-100 immunoreactivity in primary and metastatic carcinoma of the breast: A potential source of error in immunodiagnosis. Hum Pathol 1988;19:949–953.
12. Isobe T, Ishioka N, Masuda T, et al.: A rapid separation of S100 subunits by high performance liquid chromatography: The subunit compositions of S100 proteins. Biochem Int 1983;6:419–426.
13. Nakamura M, Tastuno H, Hosoda S, Saitoh Y: Immunohistochemical studies on myoepithelial cells in breast tumors. Jpn J Breast Cancer 1986;1:129–136.
14. Ohuchi N, Abe R, Takahashi T, Tezuka F: Origin and extension of intraductal papillomas of the breast. Breast Cancer Res Treat 1984;4:117–128.
15. Ohuchi N, Abe R, Kasai M: Possible cancerous change of intraductal papillomas of the breast: A 3-D reconstruction study of 25 cases. Cancer 1984;54:605–611.
16. Ichihara S, Koshikawa T, Nakamura S, et al.: Epithelial hyperplasia of usual type expresses both S100-α and S100-β in a heterogeneous pattern but ductal carcinoma in situ can express only S100-α in a monotonous pattern. Histopathology 1997;30:533–541.